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Structure, function and enzymatic synthesis of glucosaccharides assembled mainly by α1→6 linkages – A Review

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Abstract

A variety of glucosaccharides composed of glucosyl residues can be classified into α- and β-type and have wide application in food and medicine areas. Among these glucosaccharides, β-type, such as cellulose and α-type, such as starch and starch derivatives, both contain 1→4 linkages and are well studied. Notably, in past decades also α1→6 glucosaccharides obtained increasing attention for unique physiochemical and biological properties. Especially in recent years, α1→6 glucosaccharides of different molecular weight distribution have been created and proved to be functional. However, compared to β-type and α1→4 glucosaccharides, only few articles provide a systematic overview of α1→6 glucosaccharides. This motivated, the present first comprehensive review on structure, function and synthesis of these α1→6 glucosaccharides, aiming both at improving understanding of traditional α1→6 glucosaccharides, such as isomaltose, isomaltooligosaccharides and dextrans, and to draw the attention to newly explored α1→6 glucosaccharides and their derivatives, such as cycloisomaltooligosaccharides, isomaltomegalosaccharides, and isomalto/malto-polysaccharides.

Keywords: isomaltose; isomaltooligosaccharides; cycloisomaltooligosaccharides; isomaltomegalosaccharides; isomalto/malto-polysaccharides; dextrans
1. Introduction

Glucosaccharides made of multiple glucosyl residues are highly relevant to human daily life. They are divided into two types: α and β, according to the anomeric configuration of the hydroxyl group at C-1 forming the glucosidic linkage. In α-glucose units this is on the opposite side and in the β-glucose unit on the same side as the ring oxygen (T. Suzuki, 2008). α-glucose and β-glucose assemble into α-glucosaccharides and β-glucosaccharides, respectively.

The typical representative of β-glucosaccharides is cellulose, which has been studied very extensively. Cellulose is made from glucose connected by β1→4 linkages and hydrogen bonds and widely present in plant cell walls. It is mainly extracted by methods such as steam cooking and solvent extraction, sometimes supplemented by ultrasound to increase the yield (Ghasemi, Tsianou, & Alexandridis, 2017; Zeng, Bai, Gai, Peng, & Wang, 2015). In addition to being used in the traditional textile field, cellulose can also be used to synthesize renewable polymer membranes or gels (used for dialysis, ultrafiltration, protection of wounded tissues, drug release), as well as adsorbents (Liu, Dai, Si, & Zeng, 2018; Luo et al., 2018; G. Zhao et al., 2018; J. Zhao et al., 2015).

There are many kinds of linkages between adjacent glucosyl residues in α-glucosaccharides such as α1→1, α1→2, α1→3, α1→4 and α1→6 linkages. Two of the most studied are α1→4 and α1→6 linkages. Starch is one of the typical representatives of α-glucosaccharides and has been investigated in great depth, just like cellulose, and is a common storage in tubers and seeds. The branch positions of
starch are $\alpha 1\rightarrow 6$, linkage while the rest, which is also the majority, are $\alpha 1\rightarrow 4$ linkages. According to the average degree of polymerization (DP) or dextrose equivalent, $\alpha 1\rightarrow 4$ glucosaccharides products derived from starch can be divided into maltose syrups, dextrins, maltodextrins, cyclodextrins, and limit dextrins. They are oligosaccharides prepared by enzymes ($\alpha$-amylase (EC 3.2.1.1), $\beta$-amylase (EC 3.2.1.2), cyclodextrin glucosyltransferase (CGTase, EC 2.4.1.19), etc.), by acid hydrolysis, by high temperature hydrolysis or by a combination of these methods (Uitdehaag, van der Veen, Dijkhuizen, & Dijkstra, 2002; L. Zhang, Pang, & Zhang, 2011). Maltose syrups and maltodextrins are mostly used as thickeners, sweeteners and humectants. Dextrins can be used in the field of papermaking and tape (Yang, 2017). Cyclodextrins can be used to stabilize certain ingredients, eliminate odors and undesirable compounds, as carrier for embedding drugs, and for changing the properties of drugs (Davis & Brewster, 2004).

Compared with $\alpha 1\rightarrow 4$ glucosaccharides, the functionality of $\alpha 1\rightarrow 6$ glucosaccharides is quite different. They are dietary fibers that can adjust the composition of intestinal flora and beneficial to intestinal health (H. L. Chen, Lu, Lin, & Ko, 2001; Gu et al., 2018). Moreover, they can be used for medical purposes, such as hemodialysis, embedding and transport of drugs (F. Chen, Huang, & Huang, 2020; Shukla et al., 2014) and they can also be used to improve the water solubility of some insoluble compounds (Lang et al., 2014; Shinoki et al., 2013). $\alpha 1\rightarrow 6$ glucosaccharides have a similar classification and are classified as isomaltose, isomaltooligosaccharides (IMO$s$), cycloisomaltooligosaccharides (CIs),
isomaltomegalosaccharides (IMMs), isomalto/malto-polysaccharides (IMMPs),
dextrans and derivatives thereof, but there is no systematic overview available. In the
following the structure, function (Fig. 1) and synthetic methods of $\alpha1\rightarrow6$
glucosaccharides with different DP will be discussed in detail, which provides a
theoretical foundation for future research and development of $\alpha1\rightarrow6$
glucosaccharides.

Fig. 1 The function and structure of different $\alpha1\rightarrow6$ glucosaccharides and their derivatives (H. L.
Chen et al., 2001; Falconer, Mukerjea, & Robyt, 2011; Joe et al., 2016; A. Kimura, Hara, & Weeranuch, 2017; Lang et al., 2014; Leemhuis et al., 2014; Oguma & Kawamoto, 2003). 

A isomaltose; B isomaltooligosaccharides; C ① cycloisomaltooligosaccharides, ② cycloalternan; D ① isomaltomegalosaccharides, ② double anchor type IMMs, ③ non-reducing end-single anchor type IMMs, ④ reducing end-single anchor type IMMs. The difference between ① and ④ is that the reducing end α1→4 chain of ④ is longer; E isomalto/malto-polysaccharides. F Leuconostoc mesenteroides NRRL B-512F dextrans. * The two functions only apply to C ①.

α1→4 linkage, α1→6 linkage, α1→3 linkage, Glucosyl residue.

2. Overview of α1→6 glucosaccharides

2.1 Isomaltose

Isomaltose is formed by connecting two glucose molecules through an α1→6 linkage, and has been studied in great detail. Because existing IMOs products contain a large amount of isomaltose, the function of these two glucosaccharides greatly overlap, and will be in focus in the section 2.2 on IMOs.

As for the synthesis of isomaltose, it has been shown that 50 g/L sucrose and a high concentration of glucose can favour the reaction path by the action of dextransucrase (EC 2.4.1.5) to formation of isomaltose at a temperature of 30°C and pH 5.4, followed by using dealuminated zeolite, for which isomaltose has 15 times higher affinity than glucose, to adsorb isomaltose in the production (Berenstmeier & Buchholz, 2004). This means that normal glucosaccharides formation from sucrose by dextransucrase will be greatly inhibited and high isomaltose concentrations will be obtained. There was a similar way that dextranase (EC 3.2.1.11) and dextransucrase
(the mechanism of these two enzymes is discussed in the dextran section 2.6) were immobilized in calcium alginate, and glucose and sucrose were used as substrates to produce isomaltose (Erhardt, Jordening, & Buchholz, 2005). At low glucose: sucrose ratios, the main enzyme activity for isomaltose synthesis was that of dextranase. With the increase of glucose content, both the quantity and quality of the dextranase substrate changed, and its contribution to product formation decreased exponentially (Fig. 2A). Due to the strengthened contribution from dextransucrase, the overall product output was still increasing. This result is in accordance with the above method using only dextransucrase.

Moreover, amyloglucosidase (AG, EC 3.2.1.3) can synthesize isomaltose as shown in the scheme in Fig. 2B (Kato, Samejima, & Takahashi, 2001). When AG acts on glucose, the reaction system contains isomaltose, maltose and glucose and these three sugars can be converted into each other. The key in this system is to increase isomaltose/maltose ratio. The isomaltose/maltose ratio was raised from 7 for the free AG to 13 for immobilized AG in the swollen state of thermosensitive poly gels at 20°C. According to a previous report, the synthesis rate of isomaltose from β-glucopyranose is higher than that of α-glucopyranose (Hehre, Okada, & Genghof, 1969). When the thermosensitive poly gels are in a swelling state, the water cluster is considered to occupy surrounding isopropyl groups of the polymer. In addition, β-glucopyranose is known to replace part of the water cluster because the chair conformation of β-glucopyranose is similar to the trigymite structure of water (Ishimura & Uedaira, 1990; Uedaira, 1985). As a result, some α-glucopyranose may
be converted to β-glucopyranose in the gel. Therefore, the isomaltose/maltose ratio increased. The reaction system was heated to a temperature higher than the lower critical solution temperature after the reaction was completed, so that the thermosensitive poly gels precipitated, which was convenient for the next batch of reactions.

![Diagram](image)

**Fig. 2** A Schematic of isomaltose synthesis by dextranucrase and dextranase (Erhardt et al., 2005). The red line indicates that dextranucrase plays a major role. The black line indicates that dextranase plays a major role. B Reactions and products present in the reaction system of AG and glucose (Kato et al., 2001).

Isomaltodextranase (from *Arthrobacter globiformis*, EC 3.2.1.94) is also
commonly used, because it is able to act on dextrans and isomaltooligosaccharides, releasing isomaltose from their non-reducing ends (Sawai, Toriyama, & Yano, 1974). Dextrans not only contain $\alpha 1\rightarrow 6$ linkages, but also branches of other linkage types, and these branches will hinder the hydrolysis of isomaltodextranase (Takayanagi, Kimura, Matsui, Okada, & Chiba, 1995). The shielding effect of branching can be eliminated by mild acid pretreatment, and the maximal degree of hydrolysis can be increased from $<40\%$ to $>90\%$. The enzyme is composed of three domains: A, C, and D (Okazawa et al., 2015). Domains A, C and D are identified as a $(\beta/\alpha)_{8}$-barrel catalytic domain, an antiparallel $\beta$-structure and a carbohydrate-binding module (CBM) of family 35, respectively. There are two isomaltose molecules in the isomaltodextranase-isomaltose complex, one is bound to the subsites -2 and -1 of the active site, the other molecule is bound to domain D.

Among the four methods mentioned above, the method of combining dextranase and dextransucrase has the highest conversion rate (about 20%), and immobilization allows the enzymes to be reused (Erhardt et al., 2005). In contrast, the method of using dextransucrase alone to act on sucrose and glucose has a low conversion rate because there is no dextranase to hydrolyze IMOs (Berensmeier & Buchholz, 2004). In addition, the method focuses on the separation and adsorption of isomaltose, rather than conversion. It is worth noting that the addition of glucose led to an increase in the conversion rate of isomaltose. The method of AG acting on glucose has a low conversion rate (about 5%) (Kato et al., 2001). Although acid hydrolysis can increase the conversion rate of dextrans, this method has high energy consumption ($95^\circ C$, 4 h).
and the price of dextrans is higher than that of sucrose and glucose (Takayanagi et al., 1995). The high price of dextrans and high energy consumption make the method costly compared to other methods. Dextrans with a content of α1→6 linkages close to 100% may be a suitable substrate for isomaltodextranase, but the only commercially available dextrans are *L. mesenteroides* NRRL B-512F dextrans. In summary, using immobilized dextranase and dextransucrase to act on sucrose and glucose for conversion, and separation with dealumined zeolite may be a suitable method to obtain isomaltose with high conversion rate and purity.

### 2.2 Isomaltooligosaccharides

Isomaltooligosaccharides (IMOs) are linear α1→6 glucosaccharides consist of 2-10 glucosyl residues connected by α1→6/α1→6 and α1→4 linkages (P. Chen et al., 2018).

IMOs are water-soluble dietary fibers, that can accelerate intestinal peristalsis and carbohydrate metabolism, and avoid the accumulation of harmful intestinal waste (H. L. Chen et al., 2001). The basic difference between IMOs and “oligosaccharides” is that they cannot be effectively digested and absorbed in the human gastrointestinal tract but directly enter the large intestine, so they are *Bifidobacterium* proliferation factors (Kohmoto et al., 1988). Due to their significant proliferation effect on *Bifidobacteria*, both *in vitro* fermentation and using *in vivo* rat models showed that IMOs promoted growth of *Bifidobacteria* (Kaulpiboon, Rudeekulhamrong, Watanasatitarp, Ito, & Pongsawasdi, 2015; Ketabi, Dieleman, & Gaenzle, 2011). IMOs promote a series of physiological functions such as the conversion by
Bifidobacteria to short chain fatty acids (SCFAs) to regulate body fluid secretion and lubricate feces, and strengthen the activities of immune cells, to secrete a large amount of immune proteins, thereby enhancing the immune function of the organism (J. Li, Tan, & Mai, 2009). When IMOs are taken alone or with complex probiotic or glutamine, they can significantly increase the average birth weight, average daily food intake, average daily weight gain and concentration of serum IgG and IgM in piglets (G. Chen, 2016; Y. Chen, 2016). The formation of tooth decay is generally caused by the adherence in the oral cavity of Streptococcus and Lactobacillus producing acid that damages the teeth. Due to their special glycosidic structure, IMOs cannot be decomposed by the oral flora, which thus can prevent dental caries. One of the main components of IMOs is isomaltose which can significantly increase the content of volatile fatty acids (VFA) in the rumen of dairy cows and the milk fat percentage of milk (Guo, Hu, Jin, & Xu, 2018). In addition, isomaltose has a sweetness equivalent to 30-60% of sucrose, and low in calories, so it can be used as a sweetener instead of sucrose, and can be used as a food additive for diabetic patients and obese people (Lee, Wang, & Lin, 2008; W. F. Zhang, Li, Lu, & Yi, 2003).

IMOs could be produced by synthesis from glucose, sucrose, or oligosaccharides, or hydrolysis of relative polysaccharides. However, in the actual production of IMOs, they are mainly synthesized by treating starch with multiple enzymes (Fig. 3). The classic production method has starch as a raw material, and liquefies the starch by heat-resistant α-amylase of Bacillus licheniformis, and thereafter fungal α-amylase together with β-amylase, and pullulanase (EC 3.2.1.41) accomplishes the
saccharification of starch (Sorndech, Sagnelli, Blennow, & Tongta, 2017), which is finally treated with α-transglucosidase (EC 2.4.1.24) from Aspergillus niger to obtain IMOs. Pullulanase is a starch debranching enzyme that specifically hydrolyses α1→6 linkages at the amylopectin branch points. α-amylase and β-amylase hydrolyze the long branches and internal branches of starch, which is beneficial to the debranching by pullulanase (Nair, Singhal, & Kamat, 2007). During the synthesis process, heat-resistant α-amylase randomly cuts α1→4 linkages, which reduces the viscosity of the starch system that changes from semi-solid to liquid. After that, fungal α-amylase, β-amylase, and pullulanase almost completely converted the reaction system into maltooligosaccharides, which is beneficial to the subsequent α-transglucosidase reaction. α-transglucosidase is a hydrolase, that can continue to hydrolyze the α1→4 linkages from the non-reducing end of low-molecular-weight glucosaccharides after saccharification to produce more glucose. However, α-transglucosidase on the other hand, acts as an transglycosidase that can transfer a glucosyl residue to another glucose, maltose, isomaltose or isomaltotriose molecule, and glucosyl residues are thus connected by α1→6 linkages to generate IMOs (Goffin et al., 2011).
Fig. 3 The main action modes of heat-resistant and fungal α-amylase (αA), β-amylase (βA), pullulanase (P), and α-transglucosidase (αT) (Goffin et al., 2011; Nair et al., 2007; Sorndech et al., 2017). ① Transglycosylation by α-transglucosidase, ② Hydrolysis by α-transglucosidase. α1→4 linkage, α1→6 linkage, Glucosyl residue or glucose.

There are also some studies using whole-cell transformation: adding A. niger to maltose (Huang et al., 2019). However, this method results in low yield of IMOs (63.3%) and complicates the operation process, together making this method remaining at the laboratory level. Lactic acid bacteria or Saccharomyces cerevisiae can be used to ferment the final reaction liquid to remove glucose and maltose, but the fermentation products (such as alcohol and lactic acid) will affect the composition and flavor of the final product, and this method will also cause the loss of some IMOs. Therefore, this method stays at the laboratory level, just like whole-cell transformation (Xu, Zhang, & Duan, 2016). There is also a study using dextranase from S. mutans to hydrolyze dextrans to obtain IMOs with an average DP of 5.
(Klahan et al., 2018). In addition, the average DP of the final product can be increased by increasing the concentration of dextran. Amylomaltase (EC 2.4.1.25) has four activities: cyclization, coupling, disproportionation, and hydrolysis (Ayudhaya, Pongsawasdi, Laohasongkram, & Chaiwanichsiri, 2016). Cyclization produces cycloamylose, and coupling is the reverse reaction of cyclization. In the disproportionation, amylomaltase cuts a fragment of α1→4 glucosaccharides and connects the fragment to the non-reducing end of another α1→4 glucosaccharide by the formation of α1→4 linkages. The hydrolysis is of α1→4 linkages. IMOs can be obtained by applying α-transglucosidase, amylomaltase, and pullulanase to starch at the same time (Kaulpiboon et al., 2015). The difference between this method and the α-transglucosidase processing saccharified starch to prepare IMOs is that the yield of this method is lower and the average DP of IMOs is higher. In the reaction system of amylomaltase, α-transglucosidase and pullulanase, the main role of amylomaltase is disproportionation. Disproportionation makes the formation of longer α1→4 chains, which are more suitable for the production of IMOs with higher DP (the specific reason is similar to the reason described in section 2.5 in that the greater the molecular weight of the GTFB-ΔN substrate, the greater the molecular weight of the product).

The hydrolysis activity is extremely low (equivalent to 0.049% of the disproportionation activity), which may be the reason for the low yield of IMOs. Compared with the classic production method, this method lacks hydrolysis, which may hinder the debranching effect of pullulanase. In other words, the reaction product contains densely branched amylopectin that cannot be converted into IMOs by
\(\alpha\)-transglucosidase. Although the relative enzymatic activity of cyclization and coupling is higher than that of hydrolysis, as the reaction progresses, the substrate of cyclization is consumed by \(\alpha\)-transglucosidase, leading to the dynamic balance of cyclization and coupling in the direction of consuming cycloamylose, which makes the content of cycloamylose in the reaction product extremely low.

Because \(\alpha 1\rightarrow 6\) glucosaccharides with DP between 2-10 are defined as IMOs, the IMOs actually prepared are a mixture containing isomaltose, so they are not strictly distinguished according to their individual DP. At this stage, research on their functional validation is essentially completed, and focus is on increasing the IMOs yield, by improving the conversion rate of the \(\alpha\)-transglucosidase through immobilization and mutation, exogenous expression. In addition, it is also a feasible method to control the average DP of IMOs through the action of amylomaltase.

2.3 Cycloisomaltooligosaccharides

Cycloisomaltooligosaccharides (CIs) are a kind of cyclic isomalto-oligosaccharides formed of glucosyl residues connected through \(\alpha 1\rightarrow 6\) linkages.

The most striking function of CIs is that they can prevent dental caries. A very important element of dental caries is plaque of which 20% of the dry weight are water-insoluble glucosaccharides (Ebisu, Misaki, Kato, & Kotani, 1974; Inoue, Yakushiji, Katsuki, Kudo, & Koga, 1988). These glucosaccharides are mainly based on \(\alpha 1\rightarrow 3\), \(\alpha 1\rightarrow 4\) and \(\alpha 1\rightarrow 6\) linkages. The \(\alpha 1\rightarrow 3\) linkages are considered to contribute to water insolubility, while the \(\alpha 1\rightarrow 6\) linkages contribute to the adhesive
properties of these glucosaccharides. Oral *Streptococcus* is one kind of bacteria that causes caries (J. Chen, Wang, Zhang, Li, & Ai, 2008). It can produce glucosyltransferases which convert sucrose into glucosaccharides. Glucosaccharides and other polysaccharides work together to cause agglomeration of *Streptococcus* cells, which then easily adhere to the teeth, thereby forming tartar. *Streptococcus* turns into an anaerobic form in tartar. Due to the effects of oral *Streptococcus* and *Limosilactobacillus*, the fermentation of sugar is promoted and lactic acid is produced, which decreases the pH of the local environment to 4-5, leading to dental caries (Yue, 2007). CIs are colorless and tasteless, so adding them to foods containing sucrose will not affect the original flavor of the food. When CIs are used as substitute for sucrose, because of the α1→6 linkages, they cannot be converted by glucosyltransferases to glucosaccharides, hence reducing the formation of tartar. When mixed with sucrose, the addition of 0.1% to 0.4% CIs will lead to obvious anti-caries effect. Animal tests showed that no caries occurred in experimental animals after adding CIs to high-sugar feed (containing 35% sucrose), while the incidence of caries in the sucrose group without CIs was as high as 80-90% (Oguma & Kawamoto, 2003). On the other hand, CIs have high affinity for glucosyltransferases, which can be inhibited by CIs, preventing sucrose from being converted to glucosaccharides and hence the adhesion of *Streptococcus* on the tooth surface, thereby exerting their anti-caries effect (Jin, Zhang, Oguma, & Qian, 1997). It has been confirmed that the anti-caries effect of CIs is not affected by saliva (Oguma & Kawamoto, 2003). Notably, CIs sulfate can inhibit the interaction between the HIV coat membrane protein gp120 and the cell surface
receptor CD4 molecule, thereby preventing the HIV virus from invading cells (Tobe et al., 2000). In addition, CIs can also be used to embed β-carotene and capsaicin pigments that cannot be embedded in cyclodextrins (Oguma & Kawamoto, 2003).

The mechanism of CIs synthesis is similar to that of cyclodextrins, except that the direct substrates are α1→6 glucosaccharides. *Paenibacillus* sp. 598K is a CI-producing strain. In the presence of starch, it simultaneously induces two extracellular enzymes: a glycoside hydrolase family 66 (GH66) CIs glucosyltransferase (CITase-598k, EC 2.4.1.248) and a GH31 α-1,6-glucosyltransferase (6GT31A, EC 2.4.1) (Ichinose et al., 2017; R. Suzuki et al., 2012). CITase catalyzes intramolecular (cyclization) and intermolecular (transglycosylation, coupling) reactions and hydrolysis to produce CIs from dextran, whereas 6GT31A catalyzes transglycosylation of starch to produce linear α1→6 glucosaccharides, which can be substrates of CITase to produce CIs (Fig. 4A) (Oguma, Tobe, & Kobayashi, 1994). The role of 6GT31A can be replaced if linear α1→6 glucosaccharides can be provided to CITase-598k. From the mechanism of action, CITase is very similar to CGTase. In addition, *Paenibacillus* sp. 598K can hydrolyze all kinds of α-D-glucose linkages except α1→1, benefitting from the hydrolytic activity of 6GT31A (Ichinose et al., 2017). The main product of the synthesis process is CI7, and the largest product that can be obtained is CI17 (Funane et al., 2008; R. Suzuki et al., 2012). The radii of gyration (RG) of CI7, CI8, CI9 and CI10 determined from small angle X-ray scattering analysis are 6.7, 6.9, 7.5 and 8.3 Å, respectively (S. Suzuki et al., 2014). There is another kind of CITase from *Bacillus circulans* T-3040
(CITase-T3040, EC 2.4.1.248), of which the main product is CI$_8$ (Funane et al., 2014).

CITase-598k and CITase-T3040 show a high sequence identity of 65% (Fujimoto et al., 2017; N. Suzuki et al., 2014). In addition, they both have four domains: a catalytic ($\beta/\alpha_8$)-domain and three $\beta$-domains, one $\beta$-domain belonging to the CBM-35 family being inserted in the catalytic domain at the seventh loop of the ($\beta/\alpha_8$)-barrel. The main difference is that the second sugar-binding site of CITase-T3040 CBM-35 can be regarded as a subsite-8 that accommodates a substrate glucosyl residue in an extension of the active site on the($3/\alpha_8$)-barrel domain, which the second sugar-binding site of CITase-598k CBM-35 cannot. This may be the reason why the main products of CITase-598k and CITase-T3040 are different.

Because the thermal stability of CITase-598K is higher than that of CITase-T3040, and the $k_{cat}/K_M$ value of the former is about 1.8 fold of the latter, the CITase-598K is more versatile for the synthesis of CIs (R. Suzuki et al., 2012). It is worth mentioning that a new dextranase that can degrade CIs and dextran was recently discovered in the genome of Paenibacillus sp. 598K, whereas Bacillus circulans T-3040 does not produce CIs-degrading enzyme (Mizushima et al., 2019).

Regarding the cyclic products with $\alpha1\rightarrow6$ linkages, there is another product called cycloalternan in which $\alpha1\rightarrow6$ and $\alpha1\rightarrow3$ linkages alternate in the cyclic glucosaccharide of DP 4. The sugar is produced by the cycloalternan-forming enzyme (CAFE) also called alternanase (EC 2.4.1) of which the mode of action is shown in Fig. 4B (Cote & Biely, 1994; Cote & Robyt, 1982). This enzyme uses panose (D-Glcp-1→6-D-Glcp-1→4-D-Glc, the optimal substrate),
D-Glcp-1→6-D-Glcp-1→3-D-Glc or alternan (alternating α1→6 and α1→3 linkages) as substrate for synthesis of cycloalternan, but D-Glcp-1→3-D-Glcp-1→6-D-Glc, dextran, and isomaltotriose cannot be used as substrate. The hydroxyl group on C-3 of the glucosyl residue at the non-reducing end of the isomaltosyl group must be free for the transisomaltosylation and cyclization reactions to occur (Y. K. Kim, Kitaoka, Kiyoshi, Kim, & Cote, 2003). When alternan is used as substrate, cyclization by alternanase directly synthesizes cycloalternan (Cote & Ahlgren, 2001). It is worth noting that only the branch with the non-reducing end of α1→6 linkage can be used as the substrate of the alternate, that is, the cyclization reaction can only cut α1→3 linkages in this process. When alternanase acts on panose (or D-Glcp-1→6-D-Glcp-1→3-D-Glc), it will first hydrolyze α1→4 (or α1→3) linkages to form isomaltose, and then two isomaltoses are cyclized into cycloalternan. Combining with the fact that alternanase cannot degrade dextran and isomaltotriose, it can be concluded that alternanase can only cut α1→3 and α1→4 linkages. Since the reaction rate of panose is much higher than that of alternan and D-Glcp-1→6-D-Glcp-1→3-D-Glc, we can infer that the hydrolysis of this enzyme of α1→4 linkages in panose is much stronger than of α1→3 linkages. Alternatively, alternanase and disproportionating enzyme (DE) can be used to produce cycloalternan from maltooligosaccharides (Y. K. Kim et al., 2003). DE is a kind of glucosyltransferase that catalyzes the transfer of glucosyl residues from the non-reducing end of one maltooligosaccharide to the non-reducing end of another by formation of an α1→6 linkage. Alternanase then transfers the isomaltosyl residue to
the non-reducing end of another isomaltosyl-maltooligosaccharide to form isomaltosyl-1→3-isomaltosyl-1→4-maltooligosaccharide, and subsequently catalyzes a cyclization to produce cycloalternan.

**Fig. 4** A The mode of action of 6GT31A and CITase in the CIs production process (Ichinose et al., 2017; R. Suzuki et al., 2012). B The action modes of DE and alternanase and the structure of cycloalternan (Cote & Ahlgren, 2001; Y. K. Kim et al., 2003). ① Using maltodextrins as substrate, ② Using alternan as substrate, ③ Using panose as substrate. — α1→4 linkage,
2.4 Isomaltomegalosaccharides

According to the relevant definition, “megalosaccharides” consist of 10 to 100 monosaccharide units (Thoma, Wright, & French, 1959). Thus isomaltomegalosaccharides (IMMs) are products having from 10 to 100 glucosyl residues connected by α1→6 linkages. The structures of these polymers are similar to those of isomalto/malto-polysaccharides. The side close to non-reducing ends contain continuous α1→6 linkages, and the side close to reducing ends have several continuous α1→4 linkages, but there are no complicated branches, because they are made from simple linear maltodextrins.

Compared with other α1→6 glucosaccharides, IMMs exhibit unique and novel functions: solubilization, anti-inflammatory and immunomodulation. IMMs (average DP = 11.0) and IMO (average DP = 3.6) could enhance the absorption of quercetin glycosides (a mixture consisting of quercetin-3-O-β-D-glucoside (Q3G, 31.8%), monosaccharide (23.3%), di (20.3%) and tri to hepta D-glucose adducts with an α1→4 linkage to the D-glucose moiety of Q3G) in the small intestinal loop and the absorption was more prominently enhanced by IMMs. These enhancements were accompanied by the solubilization of Q3G in the gut luminal contents by IMMs (Shinoki et al., 2013). Using complexation with IMMs (average DP = 11.0), the aqueous solubility of ethyl red was improved substantially to the benefit of obtaining greater biodegradability by azoreductase (Lang et al., 2014). In addition, IMMs (average DP =12.6) could suppress the transport of tight junction markers in the rat...
intestine, and this suppression was chain-length dependent. This effect on tight junctions was confirmed in Caco-2 cell monolayers, a model of the human intestinal epithelium (Hara, Kume, Iizuka, Fujimoto, & Kimura, 2018). Finally, IMMs (average DP =11.0) could induce production of tumor necrosis factor α (TNFα), interleukin 6 (IL6), and nitric oxide in primary macrophages and the gene expression profile of inflammatory factors was similar to that of lipopolysaccharide-stimulated cells. An increase in TNFα production by IMMs possibly depended on the IMMs DP (Joe et al., 2016). It can be concluded from the above that IMMs have shown application prospects in solubilization, reducing the penetration of pro-inflammatory substances, and immune responses.

IMMs are prepared from maltodextrins (DP 6-7) by the transglycosylation of dextran dextrinase (DDase, EC 2.4.1.2), and the residual α1→4 linkages at the reducing end are further degraded by porcine pancreatic α-amylase. The products are fractionated using a 50-90% methanol precipitation method to remove residual IMOs or free reducing glucosaccharides, followed by removal of anions and cations by using an ion exchange resin, and drying by lyophilization. After the above steps, IMMs (average DP 11-12) and IMOs (average DP 3-4) are finally obtained. In the IMMs of average DP of 11-12, α1→6 linkages account for 78% (Lang et al., 2014). The core enzyme in this preparation step is DDase from *Gluconobacter oxydans* that catalyzes the transfer of a glucosyl residue from the non-reducing end of a dextrin molecule to the non-reducing end of another dextrin molecule, with formation of an α1→6 linkage (Fig. 5) (Hehre, 1951). There is also an IMMs with DP as high as 53,
but no detailed preparation method was disclosed (A. Kimura et al., 2017).

Fig. 5 The three action modes and products of DDase (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005a). ① α1→6 transglycosylation, ② α1→4 disproportionation, ③ α1→6 disproportionation, — α1→4 linkage, \( \alpha \rightarrow 6 \) linkage, Glucosyl residue.

The difficulties in synthesizing IMMs lie in the choice of enzyme and substrate. For example, when amylopectin is used as substrate, steric hindrance of a branch may prevent the enzyme from binding to the substrate. In addition, DDase has a corresponding minimum substrate, thus the effect of DDase on maltose is extremely poor and will not lead to synthesis of IMMs. Therefore, when maltodextrins (DP 6-7) are used as substrate, each dextrin molecule can only provide up to 4-5 glucose units to extend the α1→6 chain, which limits the molecular weight of the synthesized IMMs. The above results mean that if IMMs with different average DPs are to be prepared, substrates of different molecular weights are required. It is worth mentioning that other reactions of DDase may affect the chain length distribution of the product. The DDase used in the above method catalyses three reactions: cutting the α1→4 linkage and connecting the glucosyl residue to the acceptor molecule with
formation of either an $\alpha 1\rightarrow 6$ or an $\alpha 1\rightarrow 4$ linkage; cutting the $\alpha 1\rightarrow 6$ linkages and connecting the glucosyl residue to the acceptor by a $\alpha 1\rightarrow 6$ linkage (Naessens et al., 2005a). This method mainly uses the first catalytic reaction, but the reaction that cuts $\alpha 1\rightarrow 6$ linkages and connects the glucosyl to the acceptor by a $\alpha 1\rightarrow 6$ linkage will possibly affect the extension and DP distribution of IMMs.

Dextran glucosidase (EC 3.2.1.70) from S. mutans can hydrolyze linear $\alpha 1\rightarrow 6$ glucosaccharides. However, when Asn311 of the enzyme was replaced by alanine, glutamine, threonine, leucine or lysine, its hydrolytic activity basically disappeared and it showed only transglycosylation activity. When para-nitrophenyl-$\alpha$-glucoside (pNP-G) is used as substrate, pNP-IG4 can be obtained (Atsuo Kimura et al., 2007). This method provides ideas for the improvement of enzymes with both hydrolytic and transglycosidic activity for synthesis of IMMs.

IMMs derivatives also have the effect of enhancing the solubility of poorly soluble compounds (A. Kimura et al., 2017). When there are many glucosyl residues connected by $\alpha 1\rightarrow 4$ linkages on the reducing side of IMMs, this substance can be called reducing end-single anchor type isomaltomegalosaccharides (R-SIMMs) which does not require $\alpha$-amylase treatment during the preparation. Using R-SIMMs as raw material and adding $\alpha$-cyclodextrin, $\beta$-cyclodextrin or $\gamma$-cyclodextrin and corresponding CGTase enzymes, a double anchor type isomaltomegalosaccharides (DIMMs) can be prepared. The reducing end of DIMMs has 2-20 glucosyl residues connected by $\alpha 1\rightarrow 4$ linkages, the non-reducing end has 1-50 glucosyl residues connected by $\alpha 1\rightarrow 4$ linkages, and in the middle 10-100 glucosyl residues are
connected by $\alpha 1\rightarrow 6$ linkages. Another derivative of IMMs which is called non-reducing end-single anchor type isomaltomegalosaccharides (N-SIMMs) has an anchor sugar chain that contains 1-100 glucosyl residues connected by $\alpha 1\rightarrow 4$ linkages at the reducing end of the IMMs chain. The preparation of this glycosaccharide is very similar to that of DIMMs. Both use cyclodextrins and a corresponding CGTase, and it is possible to control the length of the $\alpha 1\rightarrow 4$ chain added to the IMMs and R-SIMMs by selecting the type and ratio of the cyclodextrins used. In addition to cyclodextrins, oligosaccharides or polysaccharides (such as starch) can be used as raw material, as long as a corresponding transglycosidase is selected. Both of these glycosaccharides have a higher solubilizing effect on flavonoids than IMMs. IMMs and their derivatives, however, have a better solubilizing effect than cyclodextrins. This is because cyclodextrins embed poorly soluble substances in the internal cavity to achieve solubilization but the solubilization by IMMs and their derivatives is not limited by the molecular size of the insoluble compound. Furthermore, they can facilitate the dissociation of poorly water-soluble compounds from their complexes.

It may be possible to remove $\alpha 1\rightarrow 6$ disproportionation and/or change the minimal substrate of DDase by mutation. The utilization rate of maltodextrin (DP 6-7) will increase, if maltose can become the smallest substrate of DDase. For the synthesis of IMMs with DP>12, it is a feasible method to use linear dextrins with higher DP as the substrate. Linear dextrins with different DP can be obtained by alcohol fractionation of debranched starch (Ranran et al., 2018). Correspondingly, IMMs and derivatives with different DP can be synthesized.
2.5 Isomalto/malto-polysaccharides

The reducing end part of isomalto/malto-polysaccharides (IMMPs) has multiple glucosyl residues connected by $\alpha1\rightarrow4$ linkages, while non-reducing end part has multiple glucosyl residues connected by $\alpha1\rightarrow6$ linkages (van der Zaal, Klostermann, Schols, Bitter, & Buwalda, 2019). The biggest difference between IMMPs and IMMs is that IMMPs have a complex branch structure.

IMMPs have shown a positive effect in promoting beneficial changes in the intestinal flora (Leemhuis et al., 2014). Because of the enrichment of $\alpha1\rightarrow6$ linkages, IMMPs cannot be digested in the upper part of the gastrointestinal tract. They most likely also escape digestion in the small intestine and enter the large intestine, where they will be fermented by the microbiota. Studies inoculating IMMPs into human feces showed that only when the $\alpha1\rightarrow4$ linkage fragments were exhausted, the $\alpha1\rightarrow6$ linkages linked fragments could be used (Gu et al., 2018). During degradation and metabolism of IMMPs, SCFAs were produced in large quantities, which overall significantly increased the microbial diversity and relative abundance of *Bifidobacteria* and *Lactobacilli*, beneficial to the intestinal health. When IMMPs were added to the diet of mice, the plasma and liver lipid as well as bile acid levels of the mice were not affected, but the stool volume increased significantly (Mistry et al., 2020). In addition, next-generation sequencing showed that the higher relative abundance of *Bacteroides, Lachnospiracea, Roseburia* and *Odoribacter* in the IMMPs group. In addition to having a beneficial effect on the intestinal flora, the high
content of α1→6 linkages makes IMMPs having good water solubility, and they can be regarded as a novel type of dietary fiber.

The existing preparation method of IMMPs is to treat starch with 4,6-α-gluconotransferase GTFB-ΔN (EC 2.4.1.B34) from *Limosilactobacillus reuteri* 121 (Leemhuis et al., 2014). Sometimes, maltooligosaccharides are also used as sole substrate to explore the action mode of the enzyme. GTFB-ΔN can cleave α1→4 linkages and connect glucose or maltooligosaccharides to the acceptor via α1→6 linkages (Fig. 6). After the GTFB-ΔN reaction, the products are treated with α-amylase to yield a mixture containing IMMPs.
Fig. 6 The action modes and products of GTFB-ΔN, GTFD, GTFA, alternansucrase, dextranase, and dextranase (Dobruchowska et al., 2012; Gong, van Leeuwen, Vafiadi, & Dijkhuizen, 2016; Klahan et al., 2018; van Leeuwen et al., 2008). α1→4 linkage, \( \alpha_1 \rightarrow 4 \) linkage, \( \alpha_1 \rightarrow 6 \) linkage, \( \alpha_1 \rightarrow 3 \) linkage, Glucosyl residue.

When high-purity maltooligosaccharides of DP 2, 3, and 7 are used as substrates in the reaction, products with DP ranges of 2-15, 2-20, and 2-35, respectively, can be obtained. However, the content of products of DP above 10 using this method is very
low (Dobruchowska et al., 2012). Thin-layer chromatography (TLC) showed that the rate of reaction between GTFB-ΔN and maltotriose is extremely slow (Kralj et al., 2011). Based on the above results, it is speculated that when maltooligosaccharides are degraded to maltotriose, for the remaining part (maltotriose) it is difficult to continue to provide glucose to extend the α1→6 chain. That is to say, the greater the molecular weight of the substrate, the larger number of glucosyl residues can be used for transglycosylation, and the greater the molecular weight of the synthesized IMMPs. The higher the percentage of amylose or linear maltodextrins in the substrate, the higher the α1→6 linkage ratio of the product being up to 90% and 92%, respectively (Bai et al., 2015; Leemhuis et al., 2014). According to these two findings and related studies, it can be inferred that the long straight chains in starch are the key to introducing α1→6 linkages and that the α1→4,6 linked glucosyl residues of branch points prevent GTFB-ΔN from introducing a linear α1→6 chain (van der Zaal, Schols, Bitter, & Buwalda, 2018). Therefore, sometimes either isoamylase (EC 3.2.1.68) or pullulanase was used to partially degrade the starch to introduce longer linear α1→6 chains (Leemhuis et al., 2014). For example, when non-debranching enzyme treated, isoamylase treated or pullulanase treated Etenia 457 (maltodextrins manufactured from potato starch) and waxy potato starch were used as substrate of GTFB-ΔN, the α1→6 linkage content of the products were 33, 83, 66% and 4, 83, 63%, respectively. This showed that isoamylase treatment leads to IMMPs with the highest content of α1→6 linkages, most probably due to its superior debranching ability. In addition, when GTFB-ΔN acts on potato starch, it can introduce long α1→6 chains (13.5 kDa)
at the reducing end of amylopectin (van der Zaal et al., 2019). Both this result and previous results indicate that when α1→6 chains are incorporated into amylopectin, they will lead to the extension of branched chains rather than being evenly distributed on each branched chain (van der Zaal et al., 2018). This fact indicates that GTFB-ΔN follows a processing mechanism in which linear α1→6 chains take precedence over linear α1→4 chains.

GTFB-ΔN is composed of domains A, B, C, and IV (Bai, Gangoiti, Dijkstra, Dijkhuizen, & Pijning, 2017). Related studies have shown that GTFB-ΔN evolved from an ancestor close to maltooligosaccharide-processing α-amylases of subfamily GH13_5. In the process of evolution, α-amylase-like binding groove partly covered by long loops forming a tunnel, and the existence of the tunnel explains why GTFB-ΔN is biased to act on linear α1→4 glucosaccharides.

DDase and GTFB-ΔN show similarities in enzymatic properties. Their main enzymatic properties is α1→6 transglycosylation and both enzymes tend to act on amylase and amylase dextrins (Leemhuis et al., 2014; Naessens et al., 2005a). The difference is that DDase shows α1→6 and α1→4 disproportionation, and GTFB-ΔN shows α1→4 hydrolysis. In addition, their optimum temperature is around 40°C, and they will quickly be inactivated when the temperature reaches 50°C, which limits their application.

There are two types of polysaccharides similar to IMMPs: GTFA reuteran and GTFD reuteran (Gangoiti et al., 2016; Meng, Dobruchowska, Gerwig, Kamerling, & Dijkhuizen, 2015). These polysaccharides also have a high content of α1→6 linkages,
but the \(\alpha_1\rightarrow6\) and \(\alpha_1\rightarrow4\) linkages exist in an alternating pattern, which is inferred from the fact that they can be degraded by pullulanase but not by dextranase. Their structures are shown in Fig. 6 and it can be seen that the GTFD reuteran has a higher ratio of branching than reuteran. They were synthesized by GTFA (EC 2.4.1.5) from *Limosilactobacillus reuteri* 121 or *Limosilactobacillus reuteri* 35-5 using sucrose and GTFD (EC 2.4.1.B34) from *Azotobacter chroococcum* NCIMB 8003 using starch as substrate, respectively. The functions of reuteran have not been fully explored, but reuteran has been reported to be a potential probiotic that can increase the content of SCFAs (Ming et al., 2015). As to discontinuous structures, when GTFB-\(\Delta N\) acts on maltodextrins, it produces
\[
\text{D-Glcp-1}\rightarrow4\text{-D-Glcp-1}\rightarrow6\text{-D-Glcp-1}\rightarrow6\text{-D-Glc-1}\rightarrow4\text{-D-Glc}
\]
and other products of similar structure (Dobruchowska et al., 2012). It can be inferred that there are occasional \(\alpha_1\rightarrow4\) linkages in the continuous \(\alpha_1\rightarrow6\) chains of IMMPs, because GTFB-\(\Delta N\) is also able to cut off maltose and maltotriose and connect these to an acceptor via \(\alpha_1\rightarrow6\) linkages.

### 2.6 Dextrans

Dextrans are \(\alpha\)-glucosaccharides with DP of more than 100 and mainly connected by \(\alpha_1\rightarrow6\) and/or \(\alpha_1\rightarrow3\) (mutan or alternan) linkages. They can be divided into three types based on their structural characteristics, type I: the main chain is a continuous \(\alpha_1\rightarrow6\) chain, with some branches at positions 2, 3, and 4; type II: the main chain has non-continuous \(\alpha_1\rightarrow6\) and \(\alpha_1\rightarrow3\) linkages; type III: the main chain is a continuous \(\alpha_1\rightarrow3\) chain, with branches at position 6 (Jeanes, Haynes, Wilham, Rankin, & Rist,
Leuconostoc mesenteroides NRRL B-512F dextrans is the most classic dextran product type and the application and synthesis of B-512F dextrans with different molecular weight will be discussed below.

**Table 1** Three types of dextrans and representatives of each type

<table>
<thead>
<tr>
<th>Type</th>
<th>Source</th>
<th>α1→6(%)</th>
<th>α1→2</th>
<th>α1→3</th>
<th>α1→4</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. mesenteroides</em></td>
<td>NRRL B-512F</td>
<td>95</td>
<td>5</td>
<td></td>
<td></td>
<td>(Cote &amp; Robyt, 1982; Dols, Remaud-Simeon,</td>
</tr>
<tr>
<td></td>
<td>NRRL B-1355</td>
<td>95</td>
<td>5</td>
<td></td>
<td></td>
<td>Willemot, Vignon, &amp; Monsan, 1998;</td>
</tr>
<tr>
<td></td>
<td>NRRL B-1299&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65</td>
<td>24-35</td>
<td>0-11</td>
<td></td>
<td>Dols, Remaud-Simeon, Willemot, Vignon, &amp;</td>
</tr>
<tr>
<td></td>
<td>NRRL B-742</td>
<td>87</td>
<td></td>
<td>13</td>
<td></td>
<td>Monsan, 1998; Dols, Remaud-Simeon,</td>
</tr>
<tr>
<td><em>L. citreum</em></td>
<td>SK24.002</td>
<td>57</td>
<td>43</td>
<td></td>
<td></td>
<td>2018; Miao et al., 2016; Remaud-Simeon et al., 2000; Tingirikari, Kothari, &amp; Goyal, 2014; Vuillemin et al., 2018</td>
</tr>
<tr>
<td><em>Oenococcus</em></td>
<td><em>kitaharae</em></td>
<td>98</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DSM17336</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Weissella</em></td>
<td><em>cibaria</em> JAG8</td>
<td>93</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. mesenteroides</em></td>
<td>NRRL B-1355</td>
<td>60</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. citreum</em></td>
<td>ABK-1</td>
<td>60</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
"S. mutans" NRRL 6715

\[ \alpha_1 \rightarrow 3 \] linkage content of dextrans differs as obtained by different separation methods, and the difference of the substrate will also affect the \( \alpha_1 \rightarrow 3 \) linkage content of dextrans. As a result, Table 1 only gives the range of the content.

The application of dextrans is closely related to their molecular weight. They are widely used in food, medicine and other fields, and have a broad market prospect (Goulas, Cooper, Grandison, & Rastall, 2004). Small molecular weight dextrans (Mw <10 kDa) can be used to prepare dextrans derivatives. For example, 6-8 kDa dextrans can form complex with iron, which is very important for the treatment of severe anemia (Hussain et al., 2013). Low molecular weight dextrans (10 kDa <Mw <100 kDa) are suitable for use as a plasma substitute (Falconer et al., 2011; Shukla et al., 2014). Regarding medical dextrans products, the mature clinically applied products are dextrans-70 (about 70 kDa), dextrans-40 (about 40 kDa), and dextrans-20 (about 20 kDa). Dextran-70 is one of the finest plasma substitutes currently recognized in the world. When dissolved in water, it can form a certain colloidal solution and its colloidal properties are similar to those of blood plasma. For example, the osmotic pressure and viscosity of physiological saline containing 6% dextrans-70 are the same as those of blood plasma. Therefore, it has the effect of expanding the blood volume and maintaining the blood pressure (Liang, 2012). The effects of dextrans-40 and dextrans-20 are similar to those of dextrans-70, but due to their smaller molecular weight and faster excretion, their capacity to expand is not lasting. They can cover the
surface of red blood cells, make it difficult for red blood cells to aggregate, and deaggregate already the aggregated red blood cells, thereby reducing blood viscosity and improving microcirculation. In practical applications, dextrans-70 is mainly used to treat hemorrhagic shock, while dextrans-40 and dextrans-20 are mainly used to prevent acute renal failure and diffuse intravascular coagulation (J. Zhang, Chang, Ma, Li, & Liang, 2018). Because dextrans with the high α1→6 linkage content ratio are not easily degraded in the body, they can maintain the effect of expanding blood volume for about 6 h (Z. Zhang, 2001). Medium molecular weight dextrans (100 kDa < Mw < 1000 kDa) are used as emulsifiers, stabilizers, and humectants in production of beverages and cakes; large molecular weight dextrans (Mw > 1000 kDa) can be used as a column filler (Bejar et al., 2013). In addition, use of dextrans in baked goods can increase the softness and swelling of loaves, and improve the bread texture (D. Li, Jia, & Yao, 2011). Adding dextrans to fructose syrup can prevent sucrose from crystallizing and they can also be used as a part of maltose substitute to make soft chocolate.

In addition to the above effects, dextrans show new potential in the medical field. Dextran can only be degraded in the large intestine, liver, kidney, and spleen, which leads to a higher oral bioavailability (Shingel, 2004). In addition, studies have shown that delivery systems with electrically neutral and hydrophilic surfaces are ideal for mucus penetration, and dextran exhibits electrical neutrality (Ensign, Schneider, Suk, Cone, & Hanes, 2012; Hu, Lu, & Luo, 2021; Shan et al., 2015). When some drugs are combined with dextrans to form conjugates, the products have reduced toxicity and
immunogenicity combined with enhanced targeting. When catechin that is used to treat pancreatic cancer is conjugated with dextrans, the targeting will be enhanced (Vittorio et al., 2014). In addition, dextrans can serve as a transport and adsorption carrier. Dextran/siRNA have good stability, which can effectively avoid being excreted by the kidney and easily reach the target cells (F. Chen et al., 2020). The dextran-coated magnetic iron oxide nanospheres act as an excellent carrier of doxorubicin and have no obvious toxicity and side effects to rats, and can inhibit the growth of rat tumors (Peng et al., 2015).

The synthesis and molecular weight regulation of dextrans is mainly achieved by adjusting the dosage of dextran sucrase and dextranase and the concentration of sucrose.

Dextran sucrase from *Leuconostoc*, *Streptococcus* or *Limosilactobacillus* is the core enzyme used in dextran production. It can cleave glucose from sucrose and link it to the acceptor by α1→6 linkages (D. Kim, Robyt, Lee, Lee, & Kim, 2003). The molecular weight of dextrans obtained when using this method is found in the range > 100 kDa (H. Chen, 2018). In addition to dextran synthesize, dextran sucrase can be used to transfer glucosyl residues from sucrose to monosaccharides and oligosaccharides which are produced by other enzymes (such as dextranase) or added to the reaction solution to obtain oligosaccharide products (Robyt & Eklund, 1983). This reaction is called acceptor reaction. There are also studies using DDase to synthesize dextrans, using short chain amylose as substrate, and the highest reported yield reached was 74% (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005b). The mechanism of DDase is
very similar to that dextransucrase, but it uses maltooligosaccharides as substrate. The viscosity of dextrans produced by dextransucrase is higher than the product of DDase.

Dextrans produced by dextransucrase are covalently linked to the enzyme, which makes such dextrans more difficult to isolate and purify. In industry, hydrochloric acid is used to hydrolyze dextrans to obtain dextrans with different molecular weights. However, acid hydrolysis will cause dextrans to contain a large amount of Cl\(^-\), which will affect the purity of the product. Moreover, the acid hydrolysis is random resulting in a molecular weight distribution and yield of dextrans that cannot be readily reproduced. In order to solve this problem, some studies have discussed the degrading effect of dextranase on dextrans prepared by dextransucrase. Dextranases are of two types: endodextranase (EC 3.2.1.11) and exodextranase (EC 3.2.1.70). Exodextranase can hydrolyze \(\alpha_1\rightarrow6\) linkages and release glucose, isomaltose or isomaltotriose from the reducing end of the dextrans, while endodextranase randomly hydrolyzes the \(\alpha_1\rightarrow6\) linkages to release glucose, isomaltose, isomaltriose, and so on (Walker & Pulkownik, 1974). Most endodextranases require multiple consecutive \(\alpha_1\rightarrow6\) linkages for catalysis. Endodextranase can play a role in improving the yield of low molecular weight dextrans and the uniformity of products in the process of preparing dextrans by dextransucrase (H. Chen, 2018). Through combination and immobilization of these two enzymes and regulation of the sucrose concentration, a series of products of different molecular weight can be obtained: IMOs, IMMs, dextrans (Gan, 2014; Y.-M. Kim, Seo, Kang, Atsuo, & Kim, 2009; Kubik, Sikora, & Bielecki, 2004).
At high sucrose concentrations (> 200 mM) and in the presence of sufficient concentrations of acceptors, formation of high molecular weight dextran is inhibited (Tanriseven & Robyt, 1993). When free dextranucrase and dextranase work together, dextranucrase synthesizes dextrans first, which is then hydrolyzed by dextranase. The hydrolysate can also serve as acceptor for dextranucrase. As dextrans are continuously hydrolyzed by dextranase, the concentration of the hydrolysis products continues to increase. When that concentration reaches a certain level, the acceptor reaction of dextranucrase will be activated. When sucrose is depleted, there is only hydrolysis by dextranase occurring, which also leads to a decrease in DP of the product. In such a process, the higher the sucrose and the dextranase concentrations, the smaller the molecular weight of the product. Under specific conditions (30°C, pH 5, 0.5 h reaction time), when the concentrations of dextranase, dextranucrase, and sucrose are 15.3, 16.3 U ml⁻¹, and 100 mg ml⁻¹, respectively, products with DP in the range between 10 and 60 can be obtained (Goulas et al., 2004). When dextranase (2.5 U ml⁻¹) and dextranucrase (2.0 U ml⁻¹) are simultaneously added to a solution with low concentration of sucrose (100 mg ml⁻¹), the obtained oligodextrans are mainly composed of a product having molecular weight of about 5 kDa (Gan, 2014). When dextranase (2.5 U ml⁻¹) is added after 12 or 18 h of reaction with dextranucrase (2.0 U ml⁻¹) products with molecular weight predominantly at 10 kDa and 20 kDa, respectively, were obtained, and when dextranucrase (3.0 U ml⁻¹) and dextranase (1.0 U ml⁻¹) are simultaneously added to a series of solutions with high sucrose concentrations (200 mg ml⁻¹, 300 mg ml⁻¹, 400 mg ml⁻¹), oligodextrans of molecular
weight from 5 kDa to 10 kDa are obtained. By definition, oligodextrans with molecular weight of 5-8 kDa and 10 kDa are also IMMs because they correspond to products of DP = 30-50 and 61, respectively. Obviously, these methods of making IMMs are not as good as the method mentioned in the previous section 2.4, because they lead to waste of fructose from sucrose.

In a study glutaraldehyde cross-linking and sodium alginate embedding were applied to immobilize dextranase, and then dextranase (0.5 Uml⁻¹) and free dextranase (4.0 Uml⁻¹) reacted with sucrose (10% (w/v)) to synthesize IMOs, which contained 55–60% isomaltose, 20% glucose as well as isomaltotriose and isomaltotetraose (10–15% of each) (Kubik et al., 2004). The genes of L. mesenteroides dextranase and Arthrobacter dextranase were ligated in the same plasmid and introduced into E. coli for expression (Y.-M. Kim et al., 2009). The resulting fusion enzyme has both endodextranase and dextranase activity. Compared to the individual enzymes, the fusion enzyme showed 150% increased endodextranase activity and 98% decreased dextranase activity, and produced linear IMOs of DP2–10 using sucrose as a sole substrate; this process was promoted by Fe²⁺, Li⁺, K⁺ and Ni²⁺. The molecular weight and amount of IMOs could be controlled by the sucrose concentration (12.5–100 mM). A reaction mixture of 100 mM sucrose produced lower DP and higher concentration of IMOs than one of 12.5 mM sucrose. This finding is consistent with the previously reported results that higher concentrations of sucrose are more favorable for the formation of low molecular weight products. In addition, the fusion enzyme gave 30-fold higher production of
IMOs than that of a mixture of equal activities of the two enzymes, dextranase and dextranucrase.

To sum up the combined effect of dextranucrase and dextranase, factors that promote the production of shorter oligosaccharides include higher concentrations of dextranase and sucrose.

Chemical modification of dextrans enables dextrans to exhibit novel characteristics and/or enhancement of original functions (Hu et al., 2021). Esterification is cleaner than other chemical modifications. It can reduce the formation of hydrogen bonds and the crystallization of dextrans. Moreover, dextran esters have long polymer chains with amino groups that can interact with negatively charged substances. Therefore, dextran esters have good melting properties and biocompatibility, and can form stable films on a variety of materials to prevent chronic inflammation (Liebert, Wotschadlo, Laudeley, & Heinze, 2011). Poly(ethylene-glycol)-alkyl dextrans can form micelles in water to encapsulate lipophilic drugs to provide protection in the gastrointestinal tract (Francis, Cristea, & Winnik, 2004; Francis, Cristea, Yang, & Winnik, 2005). Protein-dialdehyde dextrans have been developed as a carrier molecule for immunoassays (Francis et al., 2005). Dialdehyde dextrans can also strengthen the nanostructure of biopolymer-based nanocarriers and improve their stability by forming covalent bonds (Dai et al., 2016; Fuentes et al., 2004). Diethyl-aminoethyl-dextrans have been evaluated as a veterinary adjuvant for inactivated virus vaccines since the last century (Moreno-Mendieta, Guillen, Hernandez-Pando, Sanchez, & Rodriguez-Sanoja, 2017).
Although the mechanism of action is not clear, the effect of stimulating and polarizing the response to Th2 for antibody synthesis has been confirmed (Houston, Crabbs, Kremer, & Springer, 1976; Petrovsky & Cooper, 2011). Acetylated dextrans have been used to encapsulate agonists of TLR-7, which can significantly promote the expression of pro-inflammatory cytokines by macrophages (Bachelder et al., 2010). Sulfated dextrans can inhibit absorption and penetration of viruses into the host cell, and reverse transcription, thus exhibiting an inhibitory effect on enveloped viruses (Arena et al., 2006; Saadat, Khosroushahi, & Gargari, 2019). Furthermore, the cross-linked dextrans microspheres used to load tetanus toxoid exhibited low toxicity, irritation, and high retention (close to 100%) of the antigenicity of tetanus toxoid during nasal administration (Tabassi, Tafaghodi, & Jaafari, 2008). Therefore, the microspheres are considered to be an effective mucosal adjuvant and antigen delivery system (Moreno-Mendieta et al., 2017).

For dextrans derived from other strains, they also have potential applications. *Oenococcus kitaharae* DSM17330 dextrans exhibited much higher solution viscosity and non-Newtonian fluid properties than *L. mesenteroides* NRRL B-512F dextrans, which may be attributed to the higher molecular weight (> 10^9 Da) and linearity (Vuillemin et al., 2018). The emulsion stability of *Weissella cibaria* JAG8 dextrans is higher than that of sodium alginate and guar gum, indicating that the dextrans have the potential to be used as emulsifier (Tingirikari et al., 2014). *L. mesenteroides* NRRL B-1498 dextrans have corrosion resistance and can protect steel in acidic and humid environments (Finkenstadt, Cote, & Willett, 2011). *L. mesenteroides* D9909
Dextrins exhibit a spherical structure in solution and can embed hydrophobic isoflavone genistein (Semyonov, Ramon, Shoham, & Shimoni, 2014). The dextrins synthesized by the mutant of the dextran sucrase from *L. mesenteroides* NRRL B-512F can form rubber and flexible biofilms without any plasticizer addition, which indicates that the dextrins can be regarded as a new biomaterial (Irague et al., 2012). In addition to dextran sucrase, *L. mesenteroides* NRRL B-1299 can also secrete branching sucrase (EC 2.4.1.B77). This enzyme can synthesize α1→2 linkages in the presence of both sucrose and acceptor (maltose, oligodextrins, etc.), and the content of α1→2 linkages in the product can be adjusted by controlling the ratio of sucrose and acceptor (Brison et al., 2010; Dos-Lafargue, Willemot, Monsan, & Remaud-Simeon, 2001). *L. mesenteroides* NRRL B-1299 dextrins will not be hydrolyzed by human and animal digestive enzymes, and the α1→2, α1→3 linkages can greatly increase the content of SCFAs (Sarbini et al., 2011). Such properties indicate that the dextrins are potential probiotics for improving intestinal health.

In addition to the type I dextrins described above, there are several products, which belong to types II and III. The structure of the first product has alternating α1→6 and α1→3 linkages, so they are not strictly dextrins and are more suitably called alternan (Cote & Robyt, 1982). Its typical representative is *L. mesenteroides* NRRL B-1355 alternan. *L. mesenteroides* NRRL B-1355 can secrete three extracellular glucosyltransferases: GTF-1, GTF-2 (alternansucrase, EC 2.4.1.140) and GTF-3 (dextran sucrase) (Smith, Zahnley, & Goodman, 1994; Zahnley & Smith, 1995). GTF-1 synthesizes water-insoluble glucosaccharides, GTF-2 synthesizes alternan, and
GTF-3 dextrans with a similar structure to the B-512F dextrans. Alternan exhibits low viscosity, high water solubility and potential probiotic properties, which makes it possible to apply in the food industry (Gregory, L., & Cote, 1992; Miao et al., 2016). When the concentration is higher than 12.5%, the nanoparticles of *L. citreum* ABK-1 alternan disintegrate and form an opaque gel (Karan et al., 2018). The critical concentration of alternan produced by the two truncated alternansucrases (Δ3SHALT and Δ7SHALT) rose to 15 and 20%, respectively (Wangpaiboon, Pitakchatwong, Panpetch, Charoenwongpaiboon, & Pichyangkura, 2019). In addition, alternan has also been proven to promote the proliferation, migration and differentiation of human bone marrow mesenchymal stem cells (Charoenwongpaiboon et al., 2019). The above two points make it possible for alternan to be used in the fields of drug embedding and medicine. It is worth mentioning that there is also *L. mesenteroides* NRRL B-742 dextrans, which is connected by 50% α1→6 linkages and 50% α1→3 linkages, but the α1→3 linkages are all distributed in the branched chain of glucosyl residues, and α1→6 linkages are concentrated and continuously distributed to form the main chain (Remaud-Simeon et al., 2000). Therefore, it belongs to type I and not to alternan and will not be degraded by dextranase.

*Streptococci* can produce glucosaccharides composed of less than 50% α1→6 linkages and more than 50% α1→3 linkages (Hamada & Slade, 1980), where α1→6 linkages form branches on the main chain formed by continuous α1→3 linkages. These glucosaccharides are called mutans and the product is highly water-insoluble due to the presence of a high proportion of α1→3 linkages. This water-insolubility
makes them play an essential role in cariogenesis by enhancing attachment and colonization of cariogenic bacteria on the teeth surface. Mutans may be used as a carrier for encapsulation technology, bioclogging, and production of biocompatible films and fibers due to its water insolubility (Côté & Skory, 2015; X. Li, Wang, Meng, Dijkhuizen, & Liu, 2020). The typical representative is *S. mutans* NRRL 6715 (Remaud-Simeon et al., 2000). In addition, mutans produced by *S. mutan* OMZ176 can stimulate alternative pathways of the complement system in human serum to activate immunomodulatory proteins (Inal, Nagaki, Ebisu, Kato, & Kotani, 1976).

3. Conclusion and future trends

This review describes the structure and function of six types of α1→6 glucosaccharides and their derivatives, and conducts a detailed analysis and comparison of the enzymatic synthesis process and the enzymatic properties of the enzymes used. The structures of these six product types lead to classifying α1→6 glucosaccharides according to DP or structure relatively simple. The functions of α1→6 glucosaccharides with similar DP also partially overlap, although each of them have their own unique functions and applications: isomaltose and IMOs have prebiotic effect, CIs can embed some kinds of pigments that cannot be embedded by cyclodextrins, and CIs are better in preventing dental caries. IMMs show solubilizing and anti-inflammatory effects, IMMPs are more readily available water-soluble dietary fibers, because their synthesis only needs one enzyme, and finally dextrans are used in the medical field.

Currently, only isomaltose, IMOs and *L. mesenteroides* NRRL B-512F dextrans
have been commercialized. The immobilized dextransucrase and dextranase can be used to act on sucrose and glucose to obtain a high conversion rate of isomaltose, which is convenient for subsequent separation and adsorption. For the synthesis of IMOs, IMOs with higher DP can be obtained by adding amylomaltase in the classical synthesis process and directly hydrolyzing dextrans. The molecular weight of dextrans can be controlled by the ratio of dextransucrase and dextranase and the concentration of sucrose. It is worth noting that this method can obtain α1→6 glucosaccharides with a high molecular weight span: isomaltose, IMOs, IMMs and dextrans. For IMMs, CIs, IMMPs and their derivatives, the synthesis method is single, and DDase and GTFB-ΔN may be able to replace each other. In general, enzymes with glucosyl residues transfer activity are used in these synthesis processes, but the lengths of the chain introduced by the enzyme and substrate are different.

In view of the fact that linear fragments are good acceptors for most of the glucosyltransferases addressed in this review, the combination of hydrolases, which hydrolysat e are straight-chain dextrans with appropriate chain length and specific glucosyltransferases maybe the future trend in developing and exploring α1→6 glucosaccharides.

**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AG</td>
<td>Amyloglucosidase</td>
</tr>
<tr>
<td>CAFE</td>
<td>Cycloalternan-Forming Enzyme/Alternanase</td>
</tr>
<tr>
<td>CBM</td>
<td>Carbohydrate-Binding Module</td>
</tr>
<tr>
<td>CGTase</td>
<td>Cyclodextrin Glucosyltransferase</td>
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CI\textsubscript{s} & Cycloisomaltooligosaccharides \\
DDase & Dextran Dextrinase \\
DE & Disproportionating Enzyme \\
DIMMs & Double Anchor Type Isomaltomegalosaccharides \\
DP & Degree of Polymerization \\
IMMPs & Isomalto/malto-polysaccharides \\
IMMs & Isomaltomegalosaccharides \\
IMO\textsubscript{s} & Isomaltooligosaccharides \\
N-SIMMs & Non-Reducing End-Single Anchor Type Isomaltomegalosaccharides \\
RG & Radii of Gyration \\
R-SIMMs & Reducing End-Single Anchor Type Isomaltomegalosaccharides \\
SCFAs & Short Chain Fatty Acids \\
VFA & Volatile Fatty Acids \\
GH66/31 & Glycoside Hydrolase Family 66/31 \\
CITase-598K/T3040 & CI\textsubscript{s} Glucosyltransferase from \textit{Paenibacillus} sp. 598K/
\textit{Bacillus circulans} T-3040 \\
6GT31A & \(\alpha\)-1,6-Glucosyltransferase from \textit{Paenibacillus} sp. 598K \\
Q3G & Quercetin-3-O-\(\beta\)-D-Glucoside \\
TNF\(\alpha\) & Tumor Necrosis Factor \(\alpha\) \\
IL6 & Interleukin 6 \\
pNP-G/IG4 & \textit{para}-Nitrophenyl-\(\alpha\)-Glucoside/Isomaltotetraose \\
TLC & Thin-Layer Chromatography
S. *Streptococcus*

A. *Aspergillus*

L. *Leuconostoc*

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Highlights

The structure and function of α1→6 glucosaccharides are discussed.

The synthetic rules of α1→6 glucosaccharides are summarized.

The analyses of enzymes give new ideas for the synthesis of α1→6 glucosaccharides.